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Introduction



Salt pans provide ecosystems which are essential for the survival of many animal and plant species, with special emphasis on extremophile living beings, such as the halophile *Dunaliella*.

Dunaliella sp are green microalgae that have been extensively studied. They accumulate glycerol and beta-carotene in adverse environmental situations .

Furthermore, this green microalgae genus, which was first identified more than a century ago, is considered to be one of the best commercial sources of natural β -carotene.

The production of β -carotene from mass cultivation of *D. salina* in large unstirred outdoor ponds or in paddlewheel stirred raceway ponds has been accomplished in several countries such as Israel, Australia, USA and China.

Aim

Our work aims to isolate and identify microalgae of *Dunaliella spp* from three of the main salterns, from mainland Portugal: Aveiro, Castro Marim and Tavira.

Methodology

To isolate this microalgae, two different culture medium (*liquid and solid*) were used for *cultivation purposes*. Furthermore, in order to identify *Dunaliella* amongst other halophile microorganisms included in the salterns, morphological, biochemistry and also molecular biology tools were used. For instance, spectrophotometry, PCR , agarose gel electrophoresis and DNA sequencing.

Results

The diluted sampled salt mixed in with Fabregas' medium on a volumetric flask started to show a greenish color after a month on the shaker (fig 2.), which might reveal that *Dunaliella* microalgae grew. It was only after three months of continuous shaking that a considerable amount of biomass was observed.



Fig.2: Culture of *Dunaliella* in liquid medium.

In a solid medium with the same conditions, growth of this microalgae was also detected.

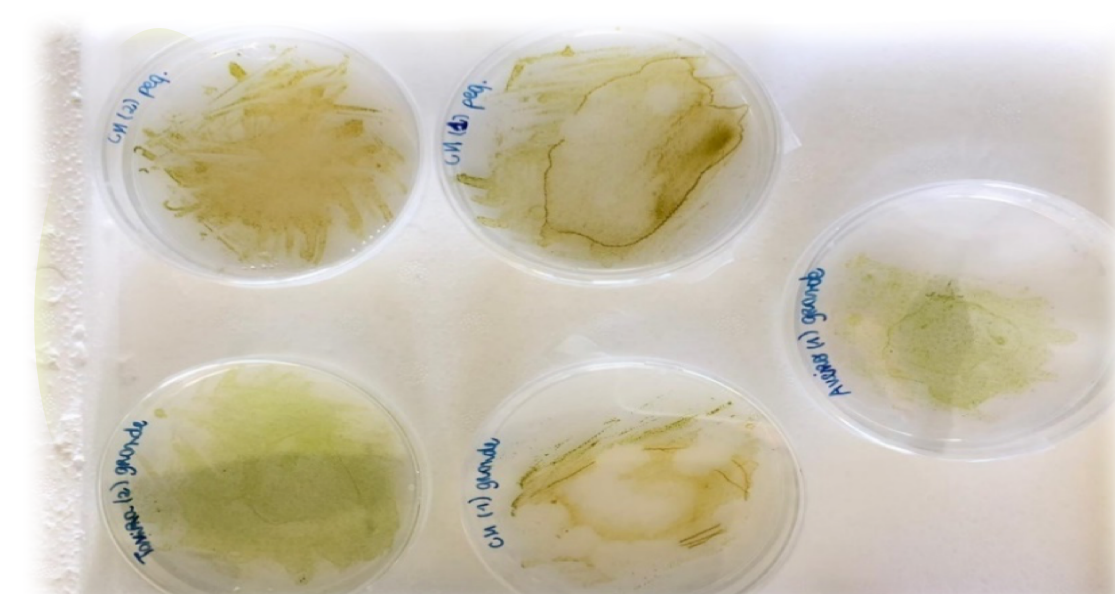


Fig.3. : Culture of *Dunaliella* in solid medium.

After six months without treatment, while some cultures remained intact, others became pink / orange, a result of the production of beta-carotene. Additionally, these had higher salinities when comparing with the green ones.

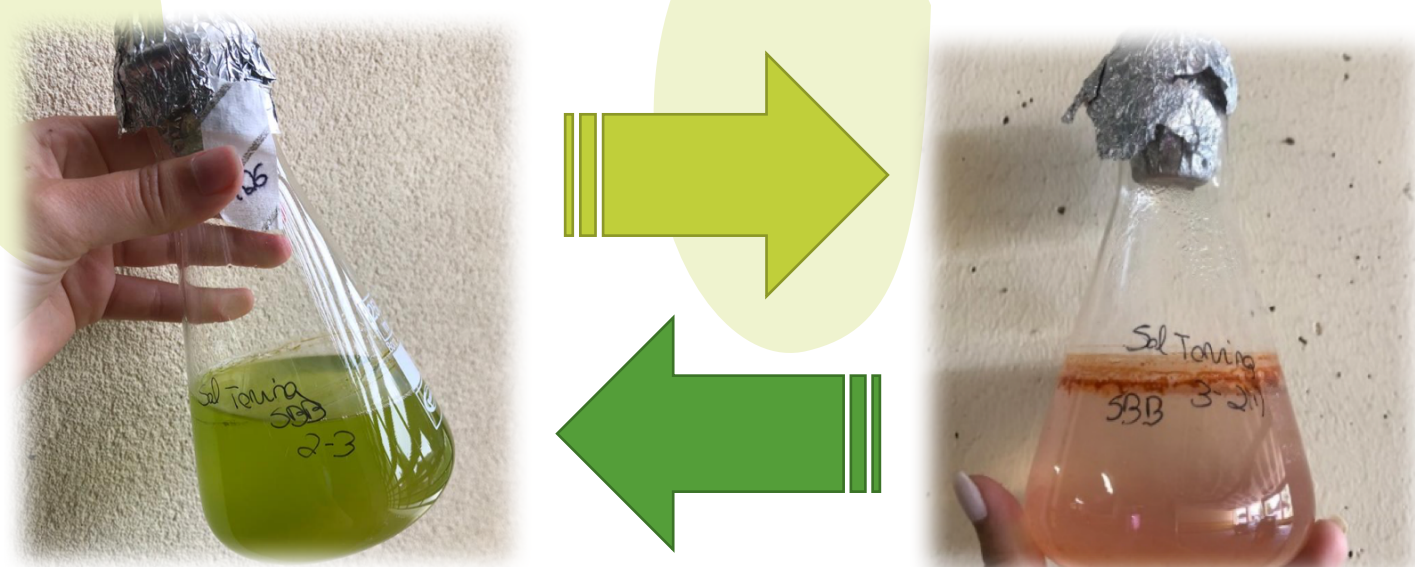


Fig.4: *Dunaliella* from Tavira in optimum conditions.

Fig.5: *Dunaliella* from Tavira after six months without treatment (Fabregas' medium).



Fig.1: *Dunaliella salina* experimental culture tanks in Abu Dhabi. Source: (Borowitzka 2018).

This algae returned to their normal state regardless of their previous situation, after their conditions returned to normal/ optimum. It is a reverseable state.

Pictures were taken while they were under the microscope, where it was possible to identify some of the common features of *Dunaliella*, such as the great variety of shapes that they display (fig.6, 7, 8), the presence of flagellate (fig.7) and sites where beta-carotene is stored (fig.8).

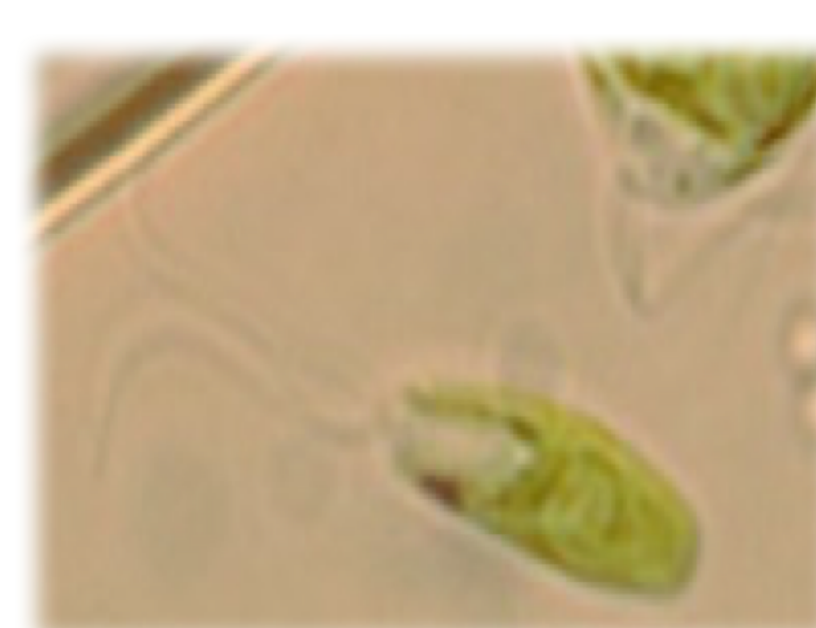


Fig.6: *Dunaliella* from young cultures from Tavira.

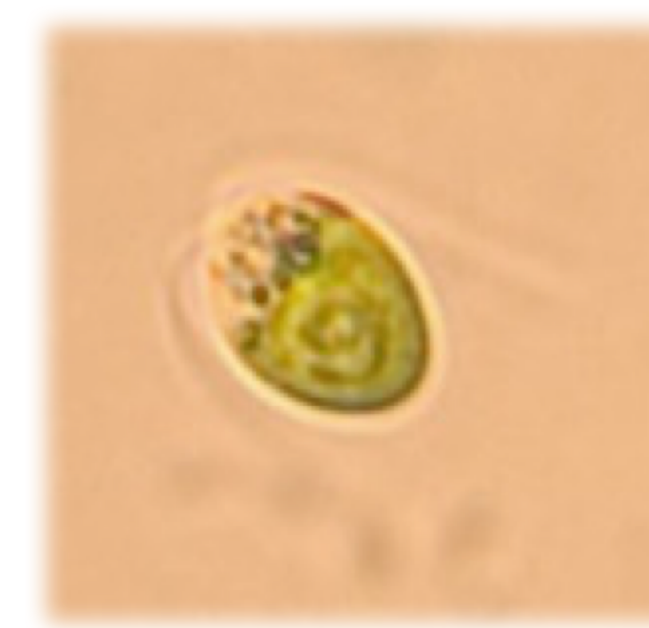


Fig.7: *Dunaliella* from young cultures from Aveiro.



Fig.8: *Dunaliella* from Castro Marim.

Conclusion

We can conclude that it is possible to create cultures of *Dunaliella* from salt crystals from salterns. Moreover, their growth in the medium is relying on optimal conditions (adequate salinity and culture medium containing the nutrients necessary for their growth). It is also possible to produce isolates of these organisms from a solid culture medium, using the same conditions.

To further complement our study, we would like to sequence the DNA of the isolates so as to identify the genus and ideally the species.

References

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